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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03000249.7

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
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targeted transgenesis using the rosa26 locus

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Targeted transgenesis using the Rosa26 locus

Introduction

The invention provides a method for targeted transgenesis using the Rosa26 locus. Suitable nucleotide acid sequences and vectors for the targeted transgenesis are provided. The Rosa26 locus proved to be a suitable integration site allowing strong and predictable expression of inserted transgenes carrying exogenous promoters.

Background of the Invention

The generation of transgenic mice by nuclear injection of purified DNA into fertilized eggs is a widely used approach for studying gene or promoter function *in vivo*. However, the level and pattern of expression often varies strongly depending on copy number, configuration, and integration site of the transgene. In addition, founder mice occasionally do not transmit the transgene. Thus, a number of different founders need to be generated and tested in order to identify a useful strain, which is a laborious and time-consuming undertaking (Bradley et. al., Nature Genet., 14:121-123 (1996); Jasin et al., Proc. Natl. Acad. Sci. USA, 93:8804-8808 (1996); Dobie et al., Trends Genet., 13:127-130 (1997); Garrick et al., Nature Genet., 18:56-59 (1998), Al-Shawi et al., Mol. Cell. Boil. 10:1192-1198 (1990)).

To overcome these limitations, homologous recombination in embryonic stem cells has been used to produce mice carrying a single copy of the transgene integrated into a predetermined site of the genome (Shaw-White et al., Transgenic Res.; (1):1-13 (1993); Bronson et al., Proc. Natl. Acad. Sci. USA, 93(17:9067-72 (1996); Hatada et al., J. Biol., Chem., 274(2):948-55 (1999); Vivian et al., Biotechniques, 27(1):154-62 (1999); Evans et al., Physiol. Genomics, Mar. 13, 2(2):67-75 (2000); Cvetkovic et al., J. boil. Chem., 275(2):1073-8 (2000); Guillot et al., Physiol. Genomics, Mar. 13, (2):77-83 (2000); Magness et al., Blood, 95(11):3568-77 (2000); Misra et al., BMC Biotechnol., 1(1):12 (2001); Minami et al., Blood, 100(12):4019-25 (2002); Tang et al., Genesis, 32(3):199-202 (2002)). In these studies, the ubiquitous Hprt locus was

more or less successfully used for 'targeted transgenesis'. Insertion of a lacZ gene under the control of the polyoma enhancer/HSV thymidine kinase promoter into the third exon of Hprt resulted in variable ß-galactosidase expression that was both orientation and cell-type dependent (Shaw-White et al., Transgenic Res.; (1):1-13 (1993)). Although transgenes under the control of the human and the chicken ß-actin gene promoter resulted in widespread expression when inserted into the Hprt locus, the level of transcripts varied strongly in different tissues (Bronson et al., Proc. Natl. Acad. Sci. USA, 93(17:9067-72 (1996)). Unexpectedly, expression of these transgenes, but not of the endogenous Hprt gene appeared to be low or undetectable in kidney and liver (Bronson et al., Proc. Natl. Acad. Sci. USA, 93(17:9067-72 (1996)). Hatada et al. demonstrated that the HPRT locus suppresses the activity of both, the haptoglobin gene promoter as well as the herpes simplex thymidine kinase promoter in several tissues of mice (Hatada et al., J. Biol., Chem., 274(2):948-55 (1999)). Likewise, a human eNOS promoter-LacZ reporter gene placed in the Hprt locus was found to be inactive in hepatic vessels that otherwise express the endogenous eNOS gene (Guillot et al., Physiol. Genomics, Mar. 13, (2):77-83 (2000). Finally, since the HPRT gene is on the X chromosome, transgene expression at this locus is subjected to random X-inactivation. The expression of the transgene in all cells of the female, therefore, requires the generation of homozygotes.

To avoid the complications referred to above, it would be desirable to define an autosomal locus that allows strong and predictable expression of transgenes inserted through homologous recombination. It is, however, not predictable for a person skilled in the art whether chromosomal loci which fulfill these criteria are available at all. Exogenous transgenes may not harbor all of the sequences necessary and sufficient for proper regulation of transcription and may therefore be influenced by cis-regulatory elements near the site of insertion.

The rosa26 locus had been identified by random insertion of retroviral sequences and a ß-galactosidase-neomycin resistance fusion gene into the genome of mouse embryonic stem cells (Zambrowicz et al., Proc. Natl. Acad. Sci. USA, 94, 3789-94 (1997)). The rosa26 promoter appeared to mediate ubiquitous expression of promoter-less genes

both in embryos and adult mice (Kisseberth et al., Dev. Biol., 214:128-138 (1999); Zambrowicz et al., Proc. Natl. Acad. Sci. USA, 94, 3789-94 (1997)), albeit at different levels in different organs (Vooijs et al., EMBO reports, 21:292-297 (2001)). However, a systematic comparison with other ubiquitous promoters to determine the strength of rosa26 promoter had not been performed. In addition, the activity of exogenous promoters inserted into the rosa26 locus has never been examined.

Summary of the Invention

The present invention is based on the finding that a particular chromosomal locus present within the eukaryotic genome (including that of mammalian ES cells), namely Rosa26, supports the preservation of the inherent activity of heterologous promoters inserted through homologous recombination at that locus. This chromosomal locus is therefore useful in the context of the "targeted transgenesis" approach for the efficient generation of transgenic organisms (such as mice) with a predictable transgene expression pattern.

Such a "targeted transgenesis" method comprises consecutive experimental steps. A gene expression cassette comprising a suitable promoter (e.g. a ubiquitous or tissue specific promoter, either inducible or constitutive) functionally linked to a gene of interest has to be created; subsequently a vector for the targeted insertion of the above mentioned gene expression cassette into the Rosa26 locus has to be generated; the insertion of the above mentioned gene expression cassette into the Rosa26 locus through homologous recombination in embryonic stem cells follows; finally transgenic mice are generated by the injection of such genetically modified ES cells into blastocysts.

More specifically present invention provides

(1) a method for generating eukaryotic cells having a modified Rosa26 locus, which method comprises the following step (hereinafter shortly referred to as step (a)): introducing a functional DNA sequence into the Rosa26 locus of starting eukaryotic cells by homologous recombination which with a targeting vector comprising said functional DNA sequence flanked by DNA sequences homologous to the Rosa26 locus;

(2) the method of (1) above, wherein the eukaryotic cells are mammalian embryonic stem (ES) cells, preferably are non-human mammalian ES cells;
(3) a targeting vector as defined in (1) above;
(4) eukaryotic cells having a modified Rosa26 locus obtainable by the method of (1) and (2) above;
(5) a method for preparing a transgenenic multi-cell organism having a modified Rosa26 locus which comprises utilizing the method as defined in (1) and (2) above;
(6) the method of (5) above, wherein the transgenenic multi-cell organism is a non-human mammal and said method comprises modifying an ES cell as defined in (2) above;
(7) a transgenic multi-cell organism and non-human mammal obtainable by the above defined methods (5) and (6), respectively; and
(8) the use of the eukaryotic cell of (4) above, the transgenic multi-cell organism of (7) above, or the transgenic non-human mammal of (7) above for gene function studies, drug development, as disease model, etc.

The method of the invention offers several advantages over the current technology of pronuclear injection. In particular, the targeting vector allows insertion of a single copy of a gene expression cassette, thus avoiding modulation of transgene expression by the arrangement of multiple copies. By choosing the autosomal Rosa26 locus as insertion site, the expression pattern of the inserted transgene in the non-human animal is predictable; random X-inactivation and/or modulation by chromosomal position effects are avoided. This also eliminates the need to generate and analyse multiple transgenic strains for any given transgene. Finally, the Rosa26 targeting vector for the site-specific integration can be used for multiple gene expression cassettes.

Description of the Figures

Figure 1: Targeted insertion of CreER and CAGGS-Cre-ER into the Rosa26 locus. A cassette comprising a Cre-ER operationally linked to a CAGGS promoter or a cassette comprising a splice acceptor site (SA) linked to a Cre-ER are inserted into the Rosa26 locus via homologous recombination. A perpendicular dash marks the insertion point

within the Rosa26 locus and the rectangular boxes delinate the starting and end points of the Rosa26 transcript.

Figure 2: Southern Blot analysis of the inducible recombination of the Rosa (reporter). (A) Genomic DNA was isolated from liver (Li) spleen (Sp) and small intestine (Si) of transgenic mice carrying the SA-creER/Rosa-rep insert or the CAGGS-creER/Rosa-rep insert. To induce the Cre-ER recombinase the mice were treated with Tamoxifen (treated). As a control, a group of mice with the SA-creER/Rosa-rep insert was left untreated (untreated). Presence of the reporter band (floxed) and deletion (deleted) of it upon an induced recombination event are indicated. (B) Transgenic mice carrying at one Rosa26 locus a loxP flanked DNA polymerase β gene segment ($pol^{\beta flox}$) and at the other a SA-creER/Rosa-rep were treated with Tamoxifen (treated). A control group of mice was left untreated (untreated). Genomic DNA from liver (Li), spleen (Sp), kidney (Ki). heart (He), lung (Lu), thymus (Th), muscle (Mu), small intestine (Si) and brain (Br) was analysed for presence of $pol^{\beta flox}$. In a non-recombination event the $pol^{\beta flox}$ band remained (floxed), in a recombination event deletion occurred (deleted). (C) As (B), but mice carried instead of the SA-creER/Rosa-rep the CAGGS-creER/Rosa-rep insert.

Figure 3: Western Blot analysis of recombinase and α-actin expression. Proteins were extracted from rosa(SA-$CreER^{T2}$) and rosa (CAGGS-$CreER^{T2}$) mice and analyzed as described in the "Materials and Method" section. The positions of bands representing $CreER^{T2}$ and actin are indicated. FA: fat tissue, Ty: Thymus; Sp: spleen, Br: Brain, Lu: lung, He: heart.

Detailed Description of the Invention

The term "living organisms" according to the present invention relates to multi-cell organisms which can be vertebrates such as mammals (e.g. non-human animals such as rodents including mice and rats; and humans) or non-mammals (e.g. fish) or can be invertebrates such as insects or worms, or can be plants (higher plants, algi or fungi). Most preferred living organisms are mice and fish.

"Eukaryotic cells" and "starting eukaryotic cells" according to the present invention include cells isolated (derived) from the above defined living organisms and cultured *in*

vitro. These cells can be transformed (immortalized) or untransformed (directl derived from living organisms; primary cell culture). The term "eukaryotic cells" also includes mono-cellular eukaryotic cells such as yeasts, etc.

It is preferred in the method (1) of the present invention that the eukaryotic cells ar derived from a multi-cell organism including vertebrates, invertebrates and plants preferably is a vertebrate cell, more preferably is derived from a mammal, including rodents such as mouse, rat, etc., or a fish such as zebrafish.

In the methods (1) and (2) of the invention it is preferred that the functional DNA sequence comprises a gene encoding a protein/peptide of interest (i.e. is a expressible and translatable DNA sequence), more preferably said functional DNA sequence is a gene expression cassette (a) comprising a gene of interest operatively linked to a promoter, or (b) is a DNA sequence which can be converted into such gene expression cassette (i.e. into an operatively linked "promoter-gene of interest" construct, e.g. by subsequent modification reactions after its integration). The gene of interest within the gene expression cassette can be any gene coding for a certain protein/peptide of interest, including, but not limited to, recombinases, reporter genes, receptors signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, disease causing gene products, toxins, etc.

The promoter of the gene expression cassette preferably is a ubiquitous or tissue specific promoter, either constitutive or inducible. Preferred ubiquitous promoter is the CAGGS-promoters are CAGGS, hCMV, PGK; preferred tissue specific promoters are FABP (Saam & Gordon, J. Biol. Chem., 274:38071-38082 (1999)), Lck (Orban et al. Proc. Natl. Acad. Sci. USA, 89:6861-5 (1992)), CamKII (Tsien et al., Cell 87: 1317-1326 (1996)), CD19 (Rickert et al., Nucleic Acids Res. 25:1317-1318 (1997)), Keratin (Li et al., Development, 128:675-88 (201)), Albumin (Postic & Magnuson, Genesis 26:149-150 (2000)), aP2 (Barlow et al., Nucleic Acids Res., 25 (1997)), Insulin (Ray et al., Int. J. Pancreatol. 25:157-63 (1999)), MCK (Brüning et al., Molecular Cell 2:559-569 (1998)), MyHC (Agak et al., J. Clin. Invest., 100:169-179 (1997), WAP (Utomo et al., Nat. Biotechnol. 17:1091-1096 (1999)), Col2A (Ovchinnikov et al., Genesis

26:145-146 (2000)); preferred inducible promoter systes are Mx (Kühn et al. Scinence, 269:1427-1429 (1995)), tet (Urlinger et al., Proc. Natl. Acad. Sci. USA, 97:7963-8 (2000)), Trex (Feng and Erikson, Human Gene Therapy, 10:419-27)

It is moreover preferred that the DNA sequences homologous to the Rosa26 locus are 0.2 to 20 kB, preferably 1 to 10 kB long. The targeting vector, functional DNA sequence or gene expression cassette may further comprises one ore more additional functional sequences including but not limited to (selectable) marker genes (such as the neomycin phosphotransferase gene of *E. coli* transposon, etc.), recombinase recognition sites (loxP, FRT, etc.) , poly A signals (such as synthetic polyadenylation sites, or the polyadenylation site of human growth hormones, etc.), splice acceptor sequences (such as a splice acceptor of adenovirus, etc.), introns, tags for protein detection, enhancers, selection markers, etc.

In a particularly preferred embodiment of the method (2) the eukaryotic cells are derived from mouse, the DNA sequences homologous to the Rosa26 locus are derived from the 5' and 3' flanking arm of the mouse Rosa26 locus, preferably said homologous DNA sequences having the sequences shown in SEQ ID NO:4 and 5, respectively, and the promoter is a CAGGS-promoter, most preferably the targeting vector has the sequence shown in SEQ ID NO:7.

The methods (1) and (2) may further (besides step (a) defined above) comprise one or more of the steps (b) isolating the eukaryotic cells, preferably the ES cells having the desired fuctional DNA sequence integrated into the Rosa26 locus; and/or (c) modifying the integrated functional DNA sequence and isolating (ES) cells having the desired modified functional DNA sequence.

The invention also provides a method for preparing a transgenenic multi-cell organism having a modified Rosa26 locus which comprises utilizing the method as defined in (1) and (2) above. This includes a method for preparing a non-human mammal comprising modifying starting ES cells according to steps (a) to (c). The ES cells may subsequently processed according one or more of the following steps:

(d) the ES cells obtained in steps (b) or (c) are injected into blastocysts; and/or
(e) transgenic non-human animals carrying one or more functional genes of interest at the Rosa26 locus are generated (viz. by well known breeding procedures).

The transgenic multi-cell organisms and non-human mammals obtainable by the method (5) and (6), respectively; preferably have an operatively functional gene expression cassette (as defined above) integrated into its Rosa26 locus. Such transgenic multi-cell organisms and non-human mammals are suitable for gene function studies, drug development, as disease model animals, etc.

The invention is further explained by the following examples and the attached figures, which are, however not to be construed so as to limit the invention.

Examples

Materials and Methods

Plasmid construction:

Rosa-targeting vector: A 129 SV/EV-BAC library (Incyte Genomics) was screened with a probe against *exon2* of the *Rosa26* locus (amplified from mouse genomic DNA using Rscreen1s (GACAGGACAGTGCTTGTTTAAGG) (SEQ ID NO:1) and Rscreen1as (TGACTACACAATATTGCTCGCAC) (SEQ ID NO:2)). Out of the identified BACclone a 11 kb EcoRV subfragment was inserted into the HindII site of pBS. Two fragments (a 1 kb SacII/XbaI- and a 4 kb XbaI-fragment) were used as homology arms and inserted into a vector consisting of a FRT-flanked neomycin resistance gene (unpublished) and a splice acceptor site from adenovirus (Friedrich G., Soriano P., Genes Dev., 5:1513-23 (1991)). The CAGGS-promoter or the splice acceptor (SA) and a synthetic intron were inserted between the 5′ arm and the neomycin resistance gene. The $CreER^{T2}$ and a polyadenylation site (pA) were cloned 3′ of the SA and CAGGS-promoter.

Cell culture: Culture and targeted mutagenesis of ES cells were carried out as previously described (Hogan et al., (Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.), pp. 253-289.) with ES cell lines derived from both inbred and F1 embryos.

Mice: All mice were kept in the animal facility at Artemis Pharmaceuticals GmbH in microisolator cages (Tecniplast Sealsave). B6D2F1 Mice for the generation of tetraploid blastocysts were obtained from Janvier. The *polb^{flox}/rosa(CreER^{T2})* and *ect2^{flox}/rosa(CreER^{T2})* mice were generated by breeding of *rosa(CreER^{T2})* ES mice with βT14 (Gu et al., *Science*, 265, 103-106.), respectively.

Production of ES mice by tetraploid embryo complementation: The production of mice by tetraploid embryo complementation was essentially performed as described (Eggan et al., *Proc Natl Acad Sci USA*, 98, 6209-6214.).

Ligand administration: 100 mg Tamoxifen-free base (Sigma, T5648) was suspended in 100 µl Ethanol and solved in 1 ml sunflower oil (Sigma). This 10 mg/100 µl tamoxifen solution was sonicated for 1-2 minutes and then stored at -20°C. For p.o. administration the solution was thawed at 55°C and administrated to 4-8 week old mice by a feeding needle (FST Fine Science Tools GmbH, 18061-20).

Western blot analysis: Western blot analysis was performed using SDS-PAGE (NuPAGE, Invitrogen) and the Breeze Immunodetection System (Invitrogen) according to the manufacturer protocols. Immunodetection was done using sc-543 (HC-20, Santa Cruz Biotechnology, Inc.) against ER, PRB-106C against cre, actin sc-1616 Actin (I-19) against actin and rabbit polyclonal IgG (Santa Cruz Biotechnology, Inc.) antibodies.

Example 1

A CreER^{T2} gene (Feil et al., (1997) *Biochem Biophys Res Commun.*, 237, 752-757) under the control of the CAGGS-promoter (Buchholz et al. (1996), *Nucl. Acids Res.*, 24, 3118-3119) was inserted into the rosa26 locus by homologous recombination in ES cells (Fig. 1). In addition, the *CreER^{T2}* gene was introduced along with a splice acceptor sequence (Friedrich and Soziano (1991), *Genes Dev.*, 9, 1513-1523) as a control for

the endogenous activity of the rosa26 gene promoter (Fig. 1). A *loxP*-flanked hygromycin resistance gene was introduced into the second allele of *rosa26* to provide test substrate for Cre ER^{T2} (Seibler et al., (2003), in press). ES cells modified at both rosa26 alleles were injected into tetraploid blastocysts and completely ES cell derived mice were generated (Eggan et al., (2001). *PNAS*, 98, 6209-6214). *Rosa(SA-CreER^{T2}/reporter)* and *Rosa(CAGGS-CreER^{T2}/reporter)* mice were fed with daily 5 mg Tamoxifen for 5 days and recombination of the reporter was analyzed 3 days after the last administration. Southern analysis of genomic DNA from different organs showed up to 50% recombination in the *Rosa(SA-CreER^{T2}/reporter)* mice and up to 90% recombination in the *rosa(CAGGS-CreER^{T2}/reporter)* mice, respectively (Fig. 2A). As the second substrate, we used the loxP flanked DNA *polymerase ß* gene segment (*polß^{flox}*) (Gu et al., (1994). *Science*, 265, 103-106). The *polß^{flox}/rosa(SA-CreER^{T2})* and *polß^{flox}/rosa(CAGGS-CreER^{T2}*mice were fed with 5 mg tamoxifen per day for 5 days and analyzed 3 days later. Southern blot analysis revealed that the *loxP*-flanked *polymerase ß* gene segment was excised in more than 90% of cells in all organs except brain in the *rosa(SA-CreER^{T2}/reporter)* mice (Fig. 2B). In contrast, the degree of inducible recombination was significantly higher in *rosa(CAGGS-CreER^{T2}/reporter)* mice, reaching 100% efficiency in most organs and up to 70% in brain.

To investigate the pattern and level of *CreER^{T2}* expression in *rosa(SA-CreER^{T2})* and *rosa(CAGGS- CreER^{T2})* mice, we performed Western analysis using antibodies specific for Cre. The 74 kDa band corresponding to the CreER^{T2} fusion protein was detectable in all organs of *rosa(CAGGS- CreER^{T2})* mice, including brain (Fig. 3). In contrast, the CreER^{T2} expression level in *rosa(SA-CreER^{T2}* mice was significantly lower compared to the *rosa(CAGGS-CreER^{T2})* strain and appeared to be undetectable in brain (Fig. 3).

SEQUENCE LISTING

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<212> DNA
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<220>
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<211> 4333
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<220>
<223> Description: 3' arm for Rosa26

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<220>
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Claims

1. Method for generating transgenic eukaryotic cells having a modified Rosa26 locus which method comprises the following step

   (a) introducing a functional DNA sequence into the Rosa26 locus of startin eukaryotic cells by homologous recombination with a targeting vecto comprising said functional DNA sequence flanked by DNA sequence homologous to the Rosa26 locus.

2. The method of claim 1, wherein the eukaryotic cells

   (i) are derived from a multi-cell organism including vertebrates invertebrates and plants, preferably are vertebrate cells, more preferabl are derived from a mammal, including rodents such as mouse, rat, etc. or a fish such as zebrafish; and/or

   (ii) are primary cells or immortalized cells;

   most preferably the cells are mammalian embryonic stem (ES) cells.

3. The method of claim 1 or 2, wherein

   (i) the functional DNA sequence is a gene expression cassette comprising a gene of interest operatively linked to a promoter or is a DNA sequence which can be converted into such gene expression cassette;and/or

   (ii) the gene of interest is selected from recombinases, reporter genes, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, disease causing gene products, toxins, etc.; and/or

   (iii) the promoter is a ubiquitous or tissue specific promoter, either constitutive or inducible, preferably is a CAGGS, hCMV, PGK, FABP, Lck, CamKII, CD19, Keratin, Albumin, aP2, Insulin, MCK, MyHC, WAP, Col2A, Mx, tet or Trex promoter; and/or

   (iv) the DNA sequences homologous to the Rosa26 locus are 0.2 to 20 kB, preferably 1 to 10 kB long; and/or

(v) the functional DNA sequence or gene expression cassette further comprises one ore more additional functional sequences including but not limited to marker genes; recombinase recognition sites, poly A signal, introns, etc.; and/or

(vi) the targeting vector further comprises tags for protein detection, enhancers, selection markers, etc.

4. The method of claim 3, wherein the transgenic eukaryotic cells are derived from mouse, the DNA sequences homologous to the Rosa26 locus are derived from the 5' and 3' flanking arm of the mouse Rosa26 locus, preferably said homologous DNA sequences having the sequences shown in SEQ ID NO:4 and 5, respectively, and the promoter is a CAGGS-promoter, most preferably the targeting vector has the sequence shown in SEQ ID NO:7.

5. The method according to any one of claims 1 to 4, which further comprises one or more of the steps

(b) isolating the eukaryotic cells, preferably the ES cells having the desired functional DNA sequence integrated into the Rosa26 locus; and/or

(c) modifying the integrated functional DNA sequence and isolating ES cells having the desired modified functional DNA sequence.

6. A targeting vector as defined in claims 1 to 4.

7. A eukaryotic cell having a modified Rosa26 locus obtainable by the method of claims 1 to 5.

8. A method for preparing transgenenic multi-cell organism having a modified Rosa26 locus which comprises utilizing the method as defined in claims 1 to 5.

9. The method of claim 8, wherein the transgenenic multi-cell organism is a non-human mammal and said method comprises modifying an ES cell as defined in claims 1 to 5.

10. The method of claim 9 which further comprises one or more of the steps
(d) injecting ES cells obtained in steps (b) or (c) into blastocysts; and/or
(e) generating transgenic non-human animals carrying one or more functional gene of interest at the Rosa26 locus.

11. A transgenic multi-cell organism and a transgenic non-human mammal obtainabl by the method of claim 8 and 9-10, respectively, and having an operativel functional gene expression cassette integrated into its Rosa26 locus.

12. Use of the eukaryotic cell of claim 7, the transgenic multi-cell organism of clair 11, or the transgenic non-human mammal of claim 11 for gene function studies drug development, as disease model animals, etc.

Abstract

The invention provides a method for targeted transgenesis using the Rosa26 locus. Suitable nucleotide acid sequences and vectors for the targeted transgenesis are provided. The Rosa26 locus proved to be a suitable integration site allowing strong and predictable expression of inserted transgenes carrying exogenous promoters.

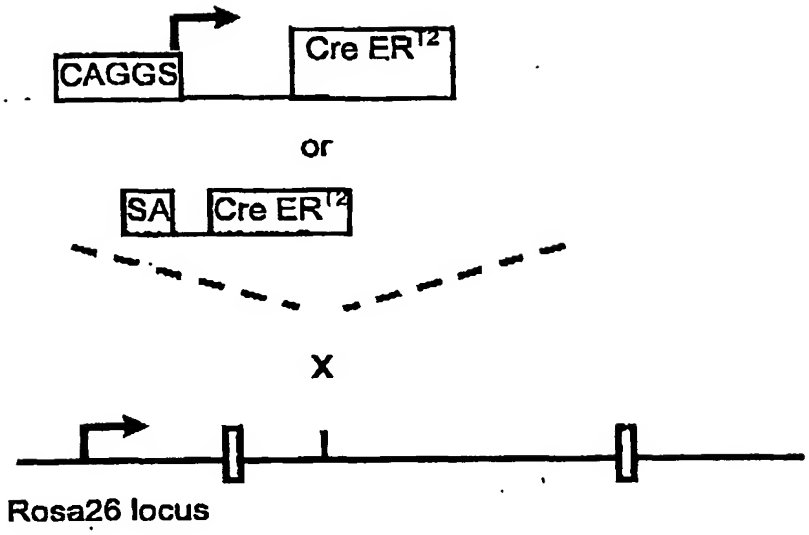
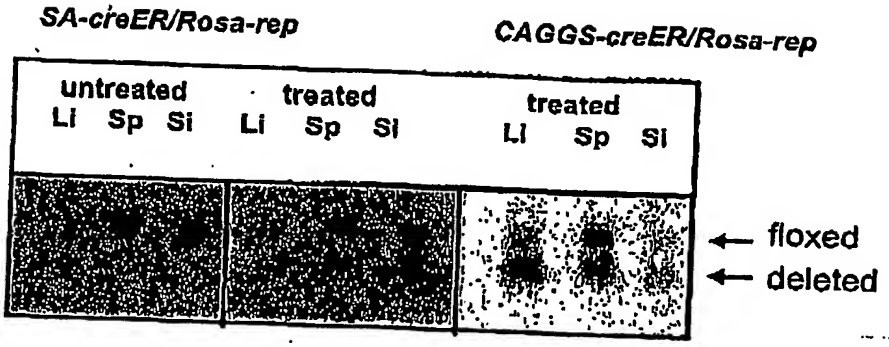


Fig.1

Fig.2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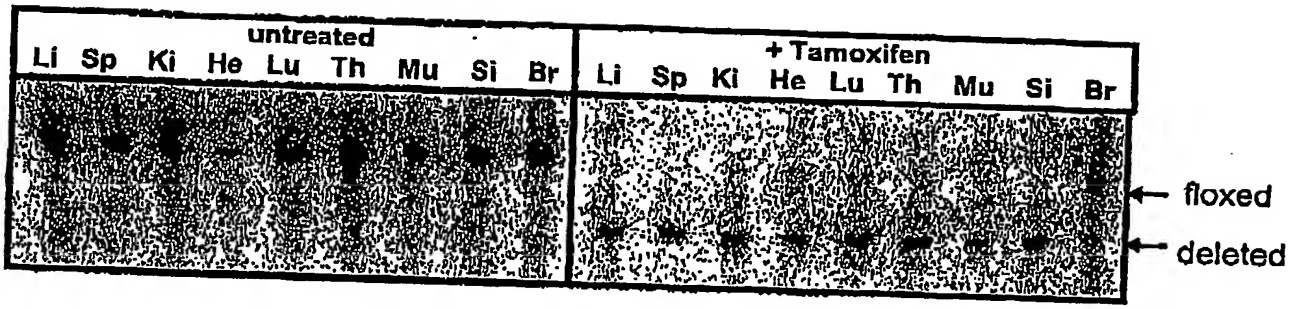
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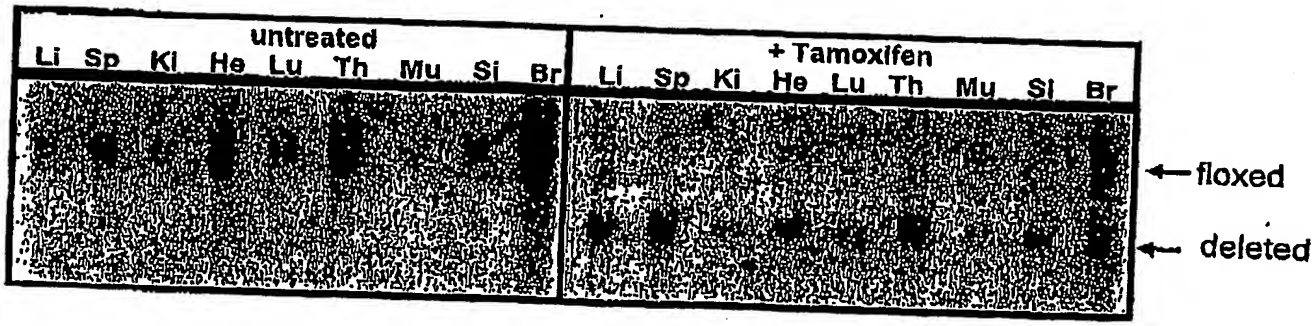
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Fig.3